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SUMMARY

The protozoan parasite, *Cryptosporidium*, has been reported as a cause of pediatric diarrhea in urban Manila, Republic of the Philippines. This study was conducted on Palawan Island to determine if *Cryptosporidium* is associated with diarrheal disease in rural areas of the Philippines. A total of 831 individuals were queried, 41 reported diarrhea and 19 of those returned stool specimens. One case of cryptosporidiosis was detected by acid-fast staining of oocysts and a string capsule test also demonstrated concurrent giardiasis. Domestic animal stools were screened for *Cryptosporidium* and one cow and carabao (water buffalo) were found positive. This study establishes the presence of *Cryptosporidium* in the rural Philippines and adds the carabao to the parasite's repertoire of hosts.

INTRODUCTION

Cryptosporidium (Protozoa; Apicomplexa), long recognized as a pathogen of veterinary importance,¹⁻³ was first reported from a human patient suffering from diarrhea⁴ in 1976. Recent literature indicates a broad and growing acceptance of this organism as a widely occurring, presumptive pathogen associated with acute and chronic diarrheal disease in immunocompromised and immunocompetent patients.⁵

The first published study of cryptosporidiosis in the Philippines reported that 2.9% of 735 stool specimens from

diarrhea patients admitted to San Lazaro Hospital, Manila, were positive for *Cryptosporidium*. All the positive cases were children 6 to 20 months of age living in or near the Metro Manila area.⁶ A second local study reported 8 patients with *Cryptosporidium*, 6 children and 2 adults (one a confirmed AIDS case).⁷

The purpose of the following study was to determine whether the parasite exists in other more remote areas of the Philippines and if so, to begin further research into its epidemiologic and immunologic characteristics.

MATERIALS AND METHODS

Study site

The study was conducted in Barangay (village) Napsan, Palawan (province) Island, Republic of the Philippines. The village is on the west coast of the island which lies about 500 miles south-south west of Manila. It is a small fishing village with a population of approximately 3,000. Housing consists primarily of bamboo-thatch huts, elevated on stilts, not closely spaced, but usually in close proximity to domestic animals. The nearest population center and health care facility is in Puerto Princesa City, about 65 k to the east across a substantial mountain range.

Collection of samples

The cryptosporidiosis study was conducted concurrently with an ongoing malaria study. As participants came in for periodic blood sampling, they were questioned about diarrhea. Of 831 individuals seen, the 41 acknowledging diarrheal episodes were given a stool cup and asked to submit a sample as soon as possible. In addition, stools were collected from local livestock and companion animals.

Preparation and examination of samples

Samples of stools returned were first concentrated by Sheathers sugar flotation, then stained by modified Kinyoun acid-fast stain and scanned by oil immersion (X1,000). Oocysts were identified as red to pink spherical bodies, about 5µm in diameter, standing out in contrast to the pale green background material. Stools positive for *Cryptospori-*

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dium were stored in 2.5% potassium dichromate and returned to our laboratory in Manila. Oocysts were then further separated by Percoll density gradient centrifugation⁶ and stored at 4°C for future use. Patients found positive for *Cryptosporidium* were sampled for duodenal fluid and epithelium by string capsule (Entero-Test, HDC Corp., Mountain View, CA). On withdrawal from the patient, the terminal 10-15 cm of the string was checked for bile stains and alkaline pH. The mucoid material on the string was stripped off using the edge of a clean microscope slide held against a petri dish. The material was applied to a slide in a drop of phosphate buffered saline, air dried, fixed in methanol, stained with Giemsa's stain, and examined under oil immersion (X1,000).

RESULTS

Of 41 stool cups handed out to patients reporting diarrhea, 19 were returned with stool samples. One specimen was positive for *Cryptosporidium* by acid-fast staining, Fig. 1. The patient was recalled. He was a 5-year old male with diarrhea of undetermined duration, 5 stools per day, which appeared dark greenish-brown, watery and of foul odor. The patient also complained of abdominal pains. Dehydration was mild, with no sign of malnutrition and no subjective indication of immunosuppression. After obtaining informed consent from the parents a string capsule was administered and left in place for four hours. After removal and examination of Giemsa stained material, the sample was found to be positive for both *Cryptosporidium* oocysts and *Giardia* trophozoites, Fig. 2. No *Giardia* trophozoites or cysts were detected in Giemsa stained fecal smears.

In addition to human stool samples, several animal stools were examined, Table 1. One calf and one young carabao (water buffalo), *Bubalus bubalis*, were found to be positive for *Cryptosporidium* oocysts by Sheathers sugar concentration and acid-fast staining, Fig. 3.



Figure 1. *Cryptosporidium* oocysts recovered from stool of 5-year old male patient from Palawan Island, Modified Kinyoun stain, X1200.



Figure 2. *Giardia* trophozoite recovered from string capsule specimen of same patient, X1200 stained with GIEMSA.

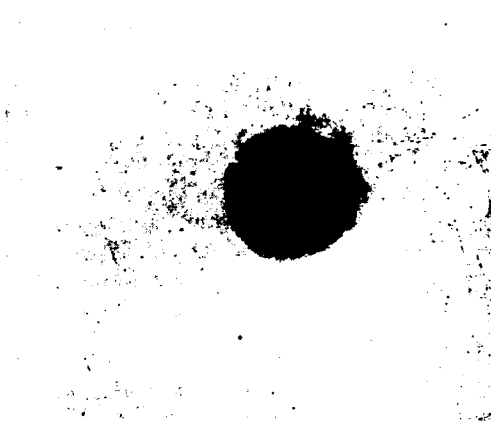


Figure 3. *Cryptosporidium* oocyst recovered from stool of carabao (water buffalo), Modified Kinyoun stain, X1200.

Table 1. Stool samples from domestic animals at Barangay Napsan, Palawan, Philippines screened for *Cryptosporidium* oocysts by sugar flotation and acid-fast stain.

Species examined	Total No.	No. Positive for <i>Cryptosporidium</i>
Bovine	1	1
Carabao	7	1
Pig	5	0
Dog	11	0
Cat	4	0
Totals	28	2

DISCUSSION

Cryptosporidiosis is a common finding in pediatric patients hospitalized for diarrhea in urban Manila, Republic of the Philippines.^{6,7} In this field study we report what we believe to be the first documented case of human cryptos-

poridiosis from Palawan Island, a rural area of the Philippines, and the first documented finding of *Cryptosporidium* in the carabao (water buffalo), *Bubalus bubalis*, a ubiquitous draft animal in the region.

Human cryptosporidiosis has been previously reported from both urban and rural communities from industrialized and developing countries.^{5,9-14} Urban areas, particularly in the developing countries, frequently experience severe overcrowding with concomitant diminished sanitation. This situation is conducive to the transmission of enteric pathogens. It would be reasonable to expect that *Cryptosporidium* oocysts are readily passed from human-to-human, human-to-animal, and animal-to-human hosts.¹⁵⁻¹⁷ In rural areas around the world, human cryptosporidiosis is putatively associated with close human-animal contact, as in the case of animal handlers and similar agricultural situations.¹⁵ The case we report here falls in the latter category. The patient, a 5-year-old male, lives in a rural community where there is no overcrowding. The family dwelling is a bamboo-thatch structure elevated on stilts. Directly around and frequently under the dwelling are a variety of domestic livestock including pigs, dogs, cats and chickens, all documented hosts of *Cryptosporidium*,² and carabao, a newly reported host. Although the exact mode of transmission of oocysts is unknown, it would again be reasonable to assume that in this community human-animal contact with subsequent hand-to-mouth transfer of oocysts is a potential source of infection.

In previous studies, some investigators reported a significant association between *Giardia* infection and *Cryptosporidium* infection in their study populations.¹⁸⁻¹⁹ Other workers reported no association between the two organisms in their respective study groups.²⁰ The patient we report here had concurrent *Giardia* and *Cryptosporidium* infections. Due to the fact that this was a preliminary study involving a very small sample size, we cannot, as yet, make inferences as to the existence of an association between these organisms in our study population. We anticipate further research in this area, as well as in transmission mode, pathology and immunology of *Cryptosporidium* in the Philippines.

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